American Journal of Medical Genetics 69:107–111 (1997)

Brief Clinical Report

Genotype-Phenotype Correlation in Two Sets of Monozygotic Twins With Williams Syndrome

Pierangela Castorina,[1] Angelo Selicorni,[2] Francesca Bedeschi,[2] Leda Dalprà,[1] and Lidia Larizza[1*]
[1]*Department of Biology and Genetics, Medical Faculty, University of Milan, Milan, Italy*
[2]*II Pediatric Department, University of Milan, Milan, Italy*

We report on two sets of monozygotic (MZ) twins with Williams syndrome (WS), following the 6 pairs already reported in the literature. We have confirmed monozygosity of both pairs of twins by DNA microsatellite analysis and the clinical diagnosis by fluorescence in situ hybridization using a WS-specific probe. Analysis of the concordance of different clinical signs between members of each pair of twins benefitted from a lengthy clinical follow-up, from 24 months to 7 years in one pair, and from the age of 15 years with reevaluation after 2 years in the other pair. Most clinical signs were concordant in the twins of each pair, with differences present at younger ages, mainly minor facial anomalies, being attenuated with time. Developmental delay was substantially concordant, but the degree differed slightly between twins in each pair. Inguinal hernia was present in a single twin in pair 1. Facial anomalies and other signs attributable to connective tissue abnormalities were also displayed by only one twin in both sets, suggesting that the WS genotype has only a predisposing role in the development of these signs. Am. J. Med. Genet. 69:107–111, 1997.

KEY WORDS: Williams syndrome; monozygotic twins; FISH; clinical signs

INTRODUCTION

Williams syndrome (WS) is a clinical condition characterized by a typical pattern of facial anomalies, developmental delay of variable severity with a friendly personality, congenital heart defect mainly consisting of supravalvular aortic stenosis. Hypercalcemia may be also present in the first years of life. Frequently associated anomalies are inguinal and/or umbilical hernia, joint hyperlaxity, possibly leading to joint limitations in adult age, kyphoscoliosis and renal/urinary tract malformations. Up to 1993, WS was considered a single-gene autosomal dominant disorder. The disease was found to be concordant in 6 sets of monozygotic (MZ) twins [Page et al., 1965; Wiltse et al., 1966; Oorthuys et al., 1984; Murphy et al., 1990; Pankau et al., 1993] and discordant in two sets of dizygotic (DZ) twins [Crichton and Morgan, 1967; Neilson and Hossack, 1978]. Although WS is usually sporadic, vertical transmission has been demonstrated in a few cases [Morris et al., 1993; Sadler et al., 1993]. Following the finding by Ewart et al. [1993] that WS is due to a submicroscopic deletion within chromosomal band 7q11.23 which involves the elastin gene and extends beyond it, fluorescence in situ hybridization (FISH) and/or genotype analysis have provided a more accurate diagnostic tool for the disorder [Nickerson et al., 1995; Lowery et al., 1995]. We report on two additional pairs of twins proven to be monozygotic by DNA fingerprinting and to be concordant for WS by FISH analysis. The spectrum of their clinical signs is compared in detail in order to discriminate between primary and secondary phenotypic manifestations.

CLINICAL REPORTS

Family 1

These male twins were born to a 28-year-old mother and a 33-year-old father at 36 weeks of gestation by caesarian section. The parents were healthy and nonconsanguineous. Birth parameters were: twin A, weight 1,740 g (<3rd centile), length 44 cm (<3rd centile), head circumference (OFC) 31 cm (<3rd centile), Apgar score 8/8; twin B, weight 2,250 g (<3rd centile), length 46 cm (<3rd centile), OFC 32 cm (<3rd centile), Apgar score 10 (5′).

In the neonatal period, twin A had an episode of hypocalcemia which was treated with intravenous infusion of Ca gluconate for three days. At 20 days he also showed a bilateral inguinal hernia which was surgically

Contract grant sponsor: MURST 40%; Contract grant sponsor: Consiglio Nazionale delle Richerche PF; Contract grant number: 41.115.19.579.

*Correspondence to: Dr. Lidia Larizza, Dipartimento di Biologia e Genetica Via Viotti 3/5-20133 Milano, Italy.

Received 11 March 1996; Accepted 11 June 1996

corrected at age 15 months. Echocardiographic evaluation showed supravalvular aortic stenosis and mild hypoplasia of pulmonary vessels in both infants at 24 months. At this age the stature of both twins was normal, weight was at the 3rd centile and OFC below the 3rd centile. When the twins were referred at 24 months to the observation of one of us (A.S.) both displayed the following minor anomalies: mild "coarseness" of the face, stellate pattern of the iris, epicanthic folds, broad nasal tip, long philtrum, thick lips, micrognathia and clinodactyly of the fifth finger (Fig. 1a and b). Twin A also had mild facial asymmetry, mild malar hypoplasia and full cheeks. Twin B showed dolichocephaly, mild periorbital fullness and a flat nasal bridge. Follow-up evaluation showed that at age 7 years, the twins' facial aspect was almost identical (Fig.1a′, b′ and a′b′). The overall clinical findings are summarized in Table I. Both twins had a moderate to severe degree of developmental retardation, which was slightly more evident in twin A. Hyperactive behavior, attention deficit disorder and language disabilities, which had been severe in early infancy, improved with age. The twins were friendly and had loquacious, outgoing personalities.

Family 2

These female twins, A and B, two of a triplet, were born to healthy, nonconsanguineous parents. Mother was 36 and father 46 years old. The pregnancy was uneventful and ended with a normal delivery at term. The third twin (C) was an unaffected male. Birth weight is the only measurement available for the triplet: A, 2,500 g (<3rd centile); B, 1,350 g (< 3rd centile); C, 2,800 g (3rd centile). Twin A had a regular neonatal period, whereas B had severe respiratory distress which required treatment in the intensive care unit. Following catheterization of the umbilical artery, B developed a prehepatic hemangioma and portal hypertension with esophageal varices. She also had an umbilical hernia. Supravalvular aortic stenosis was evident from birth in both A and B. The heart defect was surgically corrected in A when she was 17. Psychomotor development was retarded in both children who attended a regular school

Fig. 1. Family 1 twins A (right) and B (left) at 24 months (**a** and **b**), and at 7 years separate (**a′** and **b′**) and together (**a′b′**).

TABLE I. Summary of the Clinical Signs in the Two Sets of Twins With WS*

	Family 1		Family 2	
	Twin A	Twin B	Twin A	Twin B
Sex	M	M	F	F
Age (years)				
At last examination	7	7	18	18
Short stature (centile)	10th	10th	3rd	3rd
Microcephaly	+	+	+	+
"Coarse" face	−	−	+	+
Dolichocephaly	+	+	−	−
Bitemporal narrowing	+	+	−	+
Facial asimmetry	+	−	−	+
Sparse eyelashes	+	+	+	+
Strabismus	−	−	−	−
Stellate pattern of iris	+	+	−	−
Epichantal folds	+	+	+	−
Periorbital fullness	+	+	−	+
Depressed nasal bridge	−	−	−	−
Broad nasal tip	+	+	+	+
Full/sagging cheeks	+	+	−	+
Malar hypoplasia	−	−	+	−
Long philtrum	+	+	+	+
Large mouth	+	+	+	+
Thick lips	−	−	+	+
Open mouth	−	−	−	−
Maloccl/small teeth	+	+	−	−
Inguinal umbilical hernia	+	−	−	+[a]
Mental retardation	+	+	+	+
Outgoing personality	+	+	+	+
SVAS	+	+	+	+
CHD (non-SVAS)	−	−	−	−
Hypercalcemia	−	−	−	−
Renal anomalies	−	−	−	−
Kyphosis/scoliosis	−	−	−	−
Joints anomalies	−	−	−	−
Clinodactyly of fifth finger	−	−	+	+

* SVAS, supravalvolar aortic stenosis; CHD, congenital heart disease; Ing, inguinal; umb, umbilical.
[a] Umbilical artery catheterization during neonatal period.

with the support of an individual teacher. The physical growth of A was at the lower limits of normal with height lower than weight. In B weight was normal (25th centile), height at lower limits (3rd centile) and OFC below the 3rd centile. The girls were referred to one of us (A.S.) when they were 15 years old. Their mild clinical phenotype did not immediately suggest a diagnosis of WS. This was considered on the basis of previous photographic records (Fig. 2ab), and the presence of minor facial anomalies, supravalvular aortic stenosis, short stature, mild mental deficiency and friendly personality. The facial signs and overall clinical findings of both girls at a subsequent follow-up, when they were 18, are illustrated in Fig. 2a′ and b′ and detailed in Table I.

The male twin (C) had a normal neonatal growth and regular physical and psychomotor development. No heart defect was present. He attended regular school and was intellectually normal. The twins have an older sister, who is healthy.

Cytogenetic Analysis and Fluorescence In Situ Hybridization (FISH)

Prometaphase and metaphase chromosomes from PHA-stimulated lymphocyte cultures were set up and QFQ-banded according to standard procedures. Chromosomes 7 appeared structurally normal in prometaphase preparations. For in situ hybridization, a biotin-labelled probe WSCR (ONCOR, Gaithersburg, MD) was used. Hybridization and detection procedures were as recommended by the supplier. Chromosome spreads from both members of each pair of twins showed a fluorescent spot on only one chromosome 7, confirming the diagnosis of WS.

DNA Polymorphism Analysis

DNA from the patients and their parents were extracted from peripheral blood according to standard methods. Polymorphic DNA sequences D1S80, ApoB, YNZ22, IVS38TG53.0 and Cyp19 were typed using polymerase chain reaction (PCR)-based genotypic analyses. Following amplification with specific primers, the PCR products were run on 2% agarose gels (D1S80, ApoB, YNZ22) and stained with ethidium bromide. For the other markers (IVS38TG53.0 and Cyp19), one primer was labelled with γATP^{33}. (Amersham, Illinois) and PCR products were separated on 6% polyacrylamide gels. An identical pattern was observed at all loci, consistent with monozygosity for each pair of twins.

Fig. 2. Family 2 twins A (left) and B (right) at 24 months (**ab**) and at 18 years (**a′** and **b′**).

DISCUSSION

FISH analysis using the WS-specific probe provides a valuable tool in the diagnosis of WS, as molecular cytogenetic deletions have been identified in 96% of patients with classic WS [Lowery et al., 1995]. We report on the first two sets of MZ WS twins whose clinical diagnosis was confirmed by FISH. The monozygosity of twins in each pair was validated by typing five polymorphic loci, and this enabled us to analyze the genotype-phenotype correlations. WS was found to occur concordantly in the 6 sets of identical twins reported before 1993, when the syndrome was thought to be a single gene disorder. However, a few clinical signs, occurring at high frequency in WS, were not found to be shared by monozygotic twins. In the pair reported by Oorthuys [1984], only one male twin had signs of supravalvular aortic stenosis, and agenesis of the left kidney was seen only in one of the female twins described by Pankau et al. [1993]. This latter report pointed to a variable expression of the syndrome, mainly on the basis of a somewhat different facial appearance of the twins at the age

of 38 months. All clinical findings were concordant in one set of MZ twins described by Murphy et al. [1990], but elevated serum-ionized calcium, strabismus and some connective tissue abnormalities, such as prominent lips and 5th finger clinodactyly, were present only in one twin in the other set.

Our observations on two additional WS MZ twin pairs take into account the previously documented twin studies and the finding that WS is a usually sporadic, contiguous gene deletion syndrome [Ewart et al., 1993; Nickerson et al., 1995]. Although the phenotypic variability and severity of WS may be related to the size of the deletion in different patients, the pattern of primary clinical signs is likely to be the same in MZ twins. Assessement of the concordance rate in the two present sets of MZ twins was enhanced by continued clinical evaluation which allowed the characteristic facial changes and personality to be followed over time. In agreement with previous observations [Murphy et al., 1990], the differences between our twins mainly involved connective tissue abnormalities. Bilateral inguinal hernia and facial asymmetry were present only in twin A of family 1. The umbilical hernia developed by twin B in family 2 was likely to have originated from the umbilical catheterization in the neonatal period. The two twins were discordant for epicanthal folds, full cheeks, malar hypoplasia and facial asymmetry in infancy. However, these differences decreased with time. Thus, these signs may be considered manifestations of WS that do not fully correlate with the underlying genotype.

Prominent clinical traits of WS, such as typical face, cardiac defects, and abnormal cognitive profile were concordant in our pairs of twins. The differences in birth weight and degree of mental retardation are likely to be related to environmental factors. The more severe mental retardation displayed by twin B (who had a lower birth weight) in family 2 may be also linked to the neonatal distress she suffered. Conversely, it may be coincidence that the smaller co-twin A in family 1 also had more pronounced developmental retardation. Available data on the birth weight of the 6 WS twins reported in the literature allowed us to find only one case of discordance comparable to that observed in our two twin sets [Murphy et al., 1990]. However, no consistent difference in the clinical findings of the co-twins could be appreciated. In conclusion, our results confirm the concordance of WS phenotype in monozygotic twins. The variable expression reported by Pankau et al. [1993] in their set of monozygotic twins is, in our opinion, restricted to clinical manifestations for which the WS genotype acts as predisposing factor. An appropriate example is given by the elastin-related connective tissue abnormalities in WS patients, which are not only determined by the elastin deletion.

ACKNOWLEDGMENTS

This work was supported by MURST 40%: "Applicazioni Biomediche nella Biologia Molecolare" to L.L. and by Consiglio Nazionale delle Ricerche PF "Prevention and Control Disease Factors" subproject 7, grant 41.115.19.579 to L.D. The authors thank the parents of the Williams syndrome patients here described for their cooperation. F.B. is a fellow of ASM (Associazione Studio Malformazioni).

REFERENCES

Crichton JV, Morgan JC (1967): Hypercalcemia and supravalvular aortic stenosis. Lancet 1:1104.

Ewart AK, Morris CA, Atkinson D, Jin W, Sternes K, Spallone P, Stock AD, Leppert M, Keating MT (1993): Hemizygosity at the elastin locus in a developmental disorder, Williams syndrome. Nature Genet 5:11–16.

Lowery MC, Morris CA, Ewart A, Brothman LJ, Zhu XL, et al (1995): Strong correlation of elastin deletions, detected by FISH, with Williams syndrome: Evaluation of 235 patients. Am J Hum Genet 57:49–53.

Morris CA, Thomas IT, Greenberg F (1993): Williams syndrome: Autosomal dominant inheritance. Am J Med Genet 47:478–481.

Murphy MB, Greenberg F, Wilson G, Hughes M, DiLiberti J (1990): Williams syndrome in twins. Am J Med Genet Suppl. 6:97–99.

Neilson G, Hossack KF (1978): Supravalvular aortic stenosis in a twin. Br Heart J 40:1190–1192.

Nickerson E, Greenberg F, Keating MT, McCaskill C, Shaffer LG (1995): Deletions of the elastin gene at 7q11.23 occur in ~90% of patients with Williams syndrome. Am J Hum Genet 56:1156–1161.

Oorthuys JFE (1984): Monozygotic tweeling met het Williams-Beuren of "elfin face" syndroom. T Kindergeneesk 52:197–200.

Page HL, Vogel JHK, Pryor R, Blount SG (1965): Unusual obervations in supravalvular aortic stenosis (abstract). Circulation 32 (suppl): 166.

Pankau R, Gosch A, Simeoni E, Wessel A (1993): Williams-Beuren syndrome in monozygotic twins with variable expression. Am J Med Genet 47:475–477.

Sadler RS, Robinson LK, Verdaasdonk KR, Gingell R (1993): The Williams syndrome: Evidence for possible autosomal dominant inheritance. Am J Med Genet 47:468–470.

Wiltse HE, Goldbloom RB, Antia AU, Ottesen OE, Rowe RD, Cooke RE (1966): Infantile hypercalcemia syndrome in twins. N Engl J Med 275:1157–1160.